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(54) Titre : METHODES DE PRODUCTION D'ARTICLES, REVETUS DE COMPOSES D'ARGENT OXYDE, EMPLOYES COMME MATERIAUX ANTIMICROBIENS DANS DES INSTRUMENTS MEDICAUX

(54) Title: PROCESSES FOR PRODUCTION OF ARTICLES COATED WITH OXIDIZED SILVER SPECIES USED AS ANTIMICROBIAL MATERIALS FOR MEDICAL DEVICES

(57) Abrégé/Abstract:
This invention relates to methods for coating a substrate with an oxidized metal species and to the products of such methods. The methods are particularly suited for coating a substrate with a very thin coating of an oxidized metal species. The methods may be used to produce coated substrates for use in many applications, including but not limited to electronics, materials engineering and medical products. In a preferred embodiment, the metal is silver and the substrates are medical products or devices. As a result, in a preferred embodiment the invention relates to methods for coating articles with oxidized silver species used as antimicrobial materials for medical devices as well as description of these materials are provided in this invention. Coating of a variety of articles to be used as medical devices as disclosed in this invention can be carried out in both acidic or alkaline solutions using a corresponding silver salt or oxide solution or slurry and an dequate oxidizing agent. These processes provide articles coated with oxidized silver species and with outstanding antimicrobial properties.

ABSTRACT

This invention relates to methods for coating a substrate with an oxidized metal species and to the products of such methods. The methods are particularly suited for coating a substrate with a very thin coating of an oxidized metal species. The methods may be used to produce coated substrates for use in many applications, including but not limited to electronics, materials engineering and medical products. In a preferred embodiment, the metal is silver and the substrates are medical products or devices.

As a result, in a preferred embodiment the invention relates to methods for coating articles with oxidized silver species used as antimicrobial materials for medical devices as well as description of these materials are provided in this invention. Coating of a variety of articles to be used as medical devices as disclosed in this invention can be carried out in both acidic or alkaline solutions using a corresponding silver salt or oxide solution or slurry and an dequate oxidizing agent. These processes provide articles coated with oxidized silver species and with outstanding antimicrobial properties.

PROCESSES FOR PRODUCTION OF ARTICLES COATED WITH OXIDIZED SILVER SPECIES USED AS ANTIMICROBIAL MATERIALS FOR MEDICAL DEVICES

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PRIOR ART

The germicidal properties of silver, even not known as such, have been utilized since the early Mediterranean cultures. It has been known since 1000 BC and possibly before that water kept in silver vessels and then exposed to light and filtered could be rendered potable. Other forms of silver have been used throughout centuries for various applications, such as coatings for prevention of beverages from spoilage or silver plates and foils in the surgical treatments of wounds and broken bones.

The lethal effects of metals towards bacteria and lower life forms was first scientifically described by von Nageli in the late nineteenth century, and phenomenon was defined as an "*olygodynamic effect*".[1] The term *olygodynamic effect* is restricted to the solutions in which the metal concentration is several orders of magnitude lower than that which would be lethal to higher organisms.

The investigation of the bacteriostatic properties of pure metals such as Fe, Mo, Cu, V, Sn, W, Au, Al, Ta, Nb, Ti, Zr, Ni, Co, Ag and Cr, has proved that Co was the only element which was inhibitory for the bacterial growth under anaerobic conditions.[2] In the aerobic conditions both Cu and Co consistently displayed inhibitory effects. Some antimicrobial effects have been seen for Ni, Fe and V. However, other metals such as Mo, W, Al, Nb, Zr, Cr and most importantly for the present invention Ag and Sn never showed any tendency to inhibit the growth of *Streptococcus mutans*.

In the case of silver metal, it was in 1920, when Acél who was the first to attribute the antimicrobial properties of silver to the liberation of Ag^+ ions from the material.[3] Gibbard reported in 1937 that pure metallic silver has no antimicrobial activity.[4] His experiments showed that if silver is cleaned mechanically with an abrasive cloth or paper it becomes inactive. If molten silver is allowed to cool in a reduction atmosphere (e.g. hydrogen), no antimicrobial activity was found. When cooling of molten silver was carried out in air, and formation of surface oxide occurred, an antimicrobial activity was observed. Similar results were found when silver metal was treated with nitric acid in an air atmosphere (dissolution and formation of an oxide layer). Based on Gibbard's results, the pure silver was devoid of activity, but surface oxidized silver was active. Silver oxide, silver nitrate and silver chloride were always active. Also, Gibbard observed that the antimicrobial properties of silver and its compounds were reduced in the presence of proteins or glucose.

Djokić investigated the behavior of silver films, e.g. physical vapor deposited, electrodeposited, electroless deposited and metallurgical in physiological saline solutions.[5] He found that an essential factor leading to an antimicrobial activity of metallic silver is a presence of Ag oxide(s) at the surface of this material. It was demonstrated that only silver films containing silver oxides (most likely Ag_2O) showed an antimicrobial activity. The behavior was attributed to the dissolution of Ag_2O from the "silver" material and formation of Ag^+ or other complexed ions which become antimicrobially active. There was no evidence that that pure metallic silver, no matter which way it was produced i.e., physical vapor deposited, electrodeposited or electroless deposited can be dissolved in physiological media, nor these materials exhibited antimicrobial activity. It should be noted that when the physical vapor deposition of silver was carried out in an atmosphere containing oxygen the resulted product, as found by the XRD analysis contained silver oxide. Consequently, these samples exhibited antimicrobial activity. Contrary, when the physical vapor deposition was carried out from an argon atmosphere (no presence of oxygen) pure metallic, nanocrystalline silver film was deposited as confirmed by the XRD analysis. However, these films did not dissolve in physiological saline solutions, nor they exhibited antimicrobial activity at all. For an in depth understanding of structural properties of silver films produced by the reactive sputtering, see the publication of Djokić et al. in the Journal of the Electrochemical Society[5] in 2001. To prove the concept that only oxidized silver species are responsible for the antimicrobial activity Djokić further oxidized pure metallic silver samples (i.e. those produced by the electrodeposition, electroless deposition, physical vapor deposition in an argon atmosphere or metallurgically). The oxidation of these

samples was carried out electrochemically in 1 M KOH solutions, using a process very well established in the art. The electrochemically oxidized silver samples were tested for the antimicrobial activity against *Pseudomonas Aeruginosa.* Clear evidence was found that the electrochemically oxidized silver samples exhibited antimicrobial activity. This work obviously shows that only oxidized silver species, **but not elemental silver** will affect the antimicrobial activity. The findings up to date show that the "nanocrystalline" or "macrocrystalline" elemental silver does not have antimicrobial activity at all. The elemental silver, either nanocrystalline or "macrocrystalline" may exhibit some antimicrobial activity if oxidized silver species are present at these surfaces or within silver metal. Only formation of silver oxide(s), carbonates or other silver salts (except silver sulfide) at the surface or within this material, which may be influenced by an exposure of elemental silver to various bases, acids or due to atmospheric corrosion may lead to an antimicrobial activity of this material.

The use of silver on chronic wounds dates back in the 17^{th} and 18^{th} centuries. In the early 19^{th} century, silver nitrate begun to be used on burns and in ophthalmology. Concentrations of the solution ranged from 0.20 to 2.5 wt. % with the weaker solutions being reserved for the children. Silver has been found to be active against a wide range of bacterial, fungal and viral pathogens. Topical treatment of acute and chronic wounds is a preferred and selective approach to the prevention of infection and healing. In order to achieve these requirements products that are used in the prevention of infections must have certain physical and chemical properties.

When used for topical dressings, silver compounds must have low solubility. This is usually achieved by choosing compounds with a relatively low solubility products (e.g. AgCl, Ag_2SO_4, Ag_2CO_3, Ag_3PO_4, Ag-oxides). Kinetics of dissolution of these compounds in neutral aqueous solutions is quite slow. This fact is very convenient for two reasons. First, a sustained release of silver ions from the silver compounds would provide a prolonged antimicrobial activity. Second, low amounts of the silver ions released into wound exudates would not give a rise to transient high tissue blood and urine levels, thus avoiding the systemic toxicity. Of course, the choice of a silver compound depends on its reactivity with the wound exudates. This reactivity would be minimized in order to achieve the desired effect of the released silver ions, i.e. their antimicrobial activity.

Besides silver nitrate, one of the most widely used topical antimicrobial materials is silver sulfadiazine.[7] This compound is synthesized from silver nitrate and sodium sulfadiazine. Silver sulfadiazine has been used in treatments of burns, leg ulcers and also as a topical antimicrobial agent in the management of infected wounds.

Products such as silver protein (argyrols) or mild silver protein are mixtures of silver nitrate, sodium hydroxide and gelatin. They are recommended for internal use and promoted as essential mineral supplements. Although there is no theoretical or practical justification for their use, this class of compounds was recommended for the treatment of diverse diseases such as cancer, diabetes, AIDS and herpes.[8]

Silver-zinc-allantoinate was formulated as a cream and represents a combination of silver, zinc and allantoin (an agent that stimulates debridement and tissue growth.[9] Although this compound exhibited a promising effect in the preliminary studies, it has not been used in clinical applications yet.

In the past few decades several topical dressings containing silver have been developed for wound care. Among these materials the following should be mentioned: Areglaes, Silverlon, Acticoat, Actisorb, Silver 220 etc.

Antimicrobial coatings and methods of forming same were patented by R.E. Burrell and L.R. Morris.[10,11] The coatings were formed by the physical vapor deposition of biocompatible metal. The preferred biocompatible metal according to this invention is silver. Further, these authors claimed that the "specific embodiments of this invention demonstrate that atomic disorder may be created in metal powders or foils by cold working and in metal coatings by depositing by vapor deposition at low substrate temperature". In their summary of the invention (USA Patent 6,238,686 B1) the authors claim that "the coating of the one or more metals or compounds to be released into solution constitutes a matrix containing atoms or molecules of different material. The presence of different atoms or molecules results in atomic disorder in the metal matrix, for instance due to different sized atoms. The different atoms or molecules may be one or more second metals, metal compounds which are co- or sequentially deposited with the first metal or metals to be released. Alternatively, the different atoms or molecules may be adsorbed or trapped from the working gas atmosphere during reactive vapor deposition".

In claim 1 (USA Patent No. 6,238,686B1), the authors disclosed "a modified material comprising one or more metals in a form characterized by sufficient atomic disorder such that material, in contact with a solvent for the material, releases atoms, ions, molecules or clusters containing at least one metal at an enhanced rate relative to its normal ordered structure". In simple scientific terms, it is unclear what would be a material characterized by "*sufficient atomic disorder*". In the nature, most of the materials would exhibit *sufficient* atomic disorder if the true atomic disorder described (by drawings or mapping) in ordinary Chemistry or Physics handbooks were *insufficiently* disordered (with a regular geometric structure or like). It seems that the authors connected the "atomic disorder" with the "enhanced rate" of release of "atoms, ions, molecules or clusters". If the term "release" further relates to a dissolution (as defined in textbooks of General Chemistry and Physics), then this dissolution should lead to the liberation of ions or molecules in solvent. When released in the solvent, these ions or molecules are usually solvated i.e. surrounded by the molecules of the solvent. It is very unlikely that atoms of a metal will be released into a solution comprising of water such as in the wound environment. If released into solution in its elemental state, metals would rather represent a relatively larger particles comprising of more than one or a few atoms. In this case the term "atom" is not specific term and it is not exactly descriptive. It is not known yet that atoms of metals can be released into aqueous solutions at pH close to neutral, e.g. pH range 6 to 8, unless the authors are referring to the colloidal types of solutions, which are usually prepared by adequate chemical reactions *in-situ*.

The physico-chemical analysis of the material produced by the method disclosed in the USA Patent 5,681,575 and which is commercially available on the market under the name Acticoat® Silver Coated Dressing has been carried out. As the XRD analysis has found, the Acticoat® Silver Coated Dressing is a composite material consisting of Ag metal, Ag_2O and Ag_2CO_3. There are also some indications, although not very clear that some other oxides in higher valent state of silver (e.g. Ag(II)) may be present in the material. As determined by the chemical analysis, the Acticoat® Silver Coated Dressing contains about 40 to 50 % pure metallic silver with the rest being oxidized silver species e.g. Ag_2O and Ag_2CO_3 which are confirmed by the XRD analysis and by the fact that these species are soluble in ammonium hydroxide (NH_4OH). The presence of Ag_2O in the Acticoat® Silver Coated Dressing is likely a consequence of the physical vapor deposition of silver from an argon atmosphere containing **oxygen**. In this way, silver oxide is formed as a result of the reactive sputtering. The presence of Ag_2CO_3 in the material is attributed to the reaction:

$$Ag_2O+CO_2=Ag_2CO_3 \qquad (1)$$

Indeed, silver oxide is susceptible to carbon dioxide. As soon as the sputtered substrate is removed from the sputtering chamber, silver oxide will react with CO_2 from air, producing in this way Ag_2CO_3.

On the other hand an exposure of the Acticoat® Silver Coated Dressing to distilled water or to physiological saline solution (0.85 % NaCl) leads to a rise in pH from neutral to 10 and even to 11. This rise in pH is a consequence of the dissolution of Ag_2O or other oxidized silver species in water. This has been clearly demonstrated in the publications of Djokic et al.[5]

It is theorized that the antimicrobial activity of the Acticoat® Silver Coated Dressing is a consequence of the presence of oxidized silver species in this material (e.g. Ag_2O and Ag_2CO_3). Ag_2O and Ag_2CO_3 can dissolve in aqueous solutions up to a certain limit, which is determined by their respective solubility products ($K_{sp,}$ (Ag_2O) = 2×10^{-8} and $K_{sp,}$ (Ag_2CO_3) = 8×10^{-12}). In this way the availability of silver ions in the solution or in wound environment could lead to the antimicrobial activity of Acticoat® Silver Coated Dressing.

In terms of crystallinity, as found by the XRD analysis, the crystalline size of silver in the Acticoat® Silver Coated Dressing is estimated between 10 and 30 nm. When the physical vapor deposition is carried out in atmosphere containing pure argon (**no presence of oxygen or other reactive gases**) only metallic silver was deposited. This metallic silver was also nanocrystalline, however it exhibited insignificant, or not at all antimicrobial activity.[5,6] This is because this metallic nanocrystalline silver did not contain oxidized silver species. It may therefore be concluded that "nano - crystallinity" of Acticoat® Silver Coated Dressing cannot be connected with the antimicrobial activity

of this material. Again, the antimicrobial activity of the Acticoat® Silver Coated Dressing is most likely a consequence of the presence of oxidized silver species (e.g. Ag_2O and Ag_2CO_3) in this material.

In the USA Patent No. 6,087,549 (2000) Flick disclosed a procedure of applying a multi-laminate wound dressing.[12] In this invention, the author uses a multi-laminate wound dressing comprising a plurality of layers of preferably silver or silver-coated fibers in a woven fabric alternating with layers of nonconductive, preferably nonmetallic fabric. The dressing material was made of three layers. The layer that was against the bacterial culture called SN was 100 % silver plated nylon woven in a pattern of called warp knit with individual 15 denier fibers. The next layer was a non-woven 2 oz fabric composed of a mixture of 25 % three denier silver plated fibers and 75 % three denier silver plated fibers and 75 % three denier rayon fibers. The third layer was a non-woven 8 oz fabric composed of a mixture of 5 % three denier silver plated fibers 95 % three denier rayon fibers. The commercially available wound dressing produced in the way disclosed in the USA Patent 6,087,549(2000) is named Silverlon.

In the USA Patent publications No. 5,211,855(1993), No. 5,676,977(1997) and No.6,436,420 (2002) Antelman teaches that the "tetrasilver tetroxide (Ag_4O_4)" which contains two monovalent and two trivalent silver ions exhibits bactericidal, fungicidal and algicidal properties.[13-15] In this way "tetrasilver tetroxide" was recommended for water treatment (USA Patent 5,211,855), and if intravenously injected at levels of about 40 ppm of human blood this compound is claimed as a method of curing AIDS (USA Patent 5,211,855). In the USA Patent No. 6,436,420B1, Antelman disclosed a method of deposition or interstitial precipitation of "tetrasilver tetroxide (Ag_4O_4)" crystals within the interstices of fibers, yarns and/or fabrics forming such articles. The interstitial precipitation of Ag_4O_4 is realized by an immersion of the article to be treated e.g. fiber, yarn or fabric in a solution containing monovalent soluble silver salt, most preferably silver nitrate. After sufficient time, the article is removed into a heated aqueous solution (at above 80 °C or preferably at above 90 °C) containing strong alkali (most preferably NaOH) and water soluble oxidizing agent (most preferably potassium persulfate) for 30 seconds to 15 minutes. After the reaction is completed, the article is removed and washed. The article, treated in this way exhibits outstanding antimicrobial resistance towards pathogens such as viruses, bacteria, yeast and algae. The article is also resistant to the ultraviolet light and maintains the antimicrobial properties after a number of launderings.

SUMMARY OF THE INVENTION

This invention relates to methods for coating a substrate with an oxidized metal species and to the products of such methods. The methods are particularly suited for coating a substrate with a very thin coating of an oxidized metal species. The methods may be used to produce coated substrates for use in many applications, including but not limited to electronics, materials engineering and medical products.

In a preferred embodiment this invention provides processes for coating an article made of textile, adhesives, foams, yarns, threads and like with oxidized silver compounds. Since these oxidized silver compounds exhibit an outstanding antimicrobial activity, coated materials with said oxidized silver compounds can be used in various medical devices for prevention of the infections. These devices may include but are not limited at wound dressings, adhesives, sutures, catheters and like. Furthermore, this invention provides unique processes for coating these devices with oxidized silver compounds from aqueous solutions in the wide range of pH, which involves reactions in the acidic or alkaline solutions. The process can be carried out, but is not limited at temperatures between 2 and 60 °C with 10 to 40 °C being the most preferable. The invention provides a process for imparting antimicrobial properties to medical devices comprising the steps which are for the acidic or alkaline conditions described in the following text.

The process steps for preferred embodiments of the invention are as follows:

I. In the acidic solutions:

a) Immersion of the article to be used as a medical device in an aqueous/alcohol solution of NaOH for a sufficient time to provide a reasonable etching and cleaning of the surface, followed by washing of the article with distilled water until a pH of 7 is attained, in order to remove residual alkali.

b) Immersion of said article to be used as a medical device in an aqueous silver salt solution. This aqueous silver salt solution may be prepared of any silver salt soluble in water with the most preferred being silver nitrate.

c) Addition of proper quantity of an oxidizing agent solution to the mixed silver salt solution containing the medical device. The oxidizing agent can be any oxidizing substance such as persulfates, permanganates, hydrogen peroxide and like, with ammonium persulfate ($(NH_4)_2S_2O_8$) being the most preferred.

d) Addition of a proper quantity of an acid to the mixed silver salt solution containing immersed medical device. The acids that can be used according to this invention are any inorganic or organic acids such as, but are not limited at HNO_3, $HClO_4$, H_3PO_4, CH_3COOH, H_2SO_4 and like.

e) Additional agitation of the medical device in the solution comprising a soluble silver salt which is $AgNO_3$, an acid such as HNO_3, $HClO_4$, H_2SO_4 or like and an oxidizing agent which is most preferably $(NH_4)_2S_2O_8$ at temperatures between 2 and 30 °C with temperatures between 10 and 25 °C being the most preferable for 2 to 40 minutes until the article is coated with a grayish, gray or black color.

f) Removing the coated medical device from the slurry and washing the said device with distilled water until pH 7.

g) Drying the article produced in the step e) at room temperature

Alternatively after the step e) the coated article is immersed in boiling water (90 to 100 °C) for 10 minutes.

II. In the alkaline solutions:

a) Immersion of the article to be used as a medical device in an aqueous/alcohol solution of NaOH for a sufficient time to provide a reasonable etching and cleaning of the surface.
b) Removing of the article into a second solution containing silver diamino complex in the concentration sufficient to adsorb the silver ions at the surface of the said article and for duration of 2 to 5 minutes. This silver diamino complex is prepared by a dissolution of any silver salt or oxide in ammonium hydroxide. This is usually achieved by an addition a proper quantity of ammonium hydroxide to any aqueous solution or suspension of silver salt or oxide until a clear colorless solution containing $[Ag(NH_3)_2]^+$ is obtained. The pH of this solution is usually in the range from 8 to 12.
c) Removing of said article without washing or rinsing into a third solution containing a strong alkali, most preferably NaOH or KOH. Under the conditions of agitation keeping the said article in this solution until a clear colorless solution is obtained and the said article is clearly dyed with a tan, gray, brown or black color depending on the desired amount of oxidized silver species. The time of contact of said article with the alkaline solution may vary, depending on temperature and silver ion concentration, but the most preferable time is 1 to 15 minutes at room temperature or 1 to 10 minutes at temperatures 40 to 60 °C.
d) Removing of said dyed article from the reaction bath c) and washing with distilled water until pH 7.
e) Drying of the article produced in the step d) at room temperature.

Alternatively, in the step c), the process may involve, depending on the amount of silver required at the surface of the said article, further additions of the silver diamino complex solution and/or further additions of the oxidizing agent namely $KMnO_4$, H_2O_2, $(NH_4)_2S_2O_8$, $K_2S_2O_8$, $Na_2S_2O_8$ but most preferably $(NH_4)_2S_2O_8$ to the solution of strong alkali.

DETAILED DESCRIPTION OF THE INVENTION

Antimicrobial properties of medical devices in the present invention are achieved by the adsorption and deposition of antimicrobially active silver species within or at the surface of such device. These active silver species may include but are not limited at all oxidized silver species such as silver salts, silver oxide (Ag_2O), higher silver oxides i.e. Ag(II) and Ag(III) (AgO, Ag_2O_3, Ag_3O_4 or like), silver oxy-salts with a general formula $Ag(Ag_3O_4)X$ were X can include one of acid anions such as sulfates, chlorides, phosphates, carbonates, citrates, tartrates, oxalates and like. The coating may also contain some elemental silver deposited during the processing.

The term "total silver" as used in this invention is the total amount of silver as determined by the chemical analysis, which may include the elemental (metallic) silver as well as silver originating from oxidized silver species.

The term "medical devices" as used herein is intended to include a wide range of the devices but is not limited at wound dressings, adhesives, sutures, catheters, threads, yarns, fabrics and similar. The term "oxidized silver species" as used in the present invention may involve but is not limited at all compounds of silver where said silver is in +I, +II or +III valent states or any combinations made therein. These oxidized silver species are for example silver (I) oxide, silver (II) oxide, silver (III) oxide or mixtures therein, all silver salts having the solubility product higher than 10^{-20}, such as for example Ag_2SO_4, AgCl, $Ag_2S_2O_8$, Ag_2SO_3, $Ag_2S_2O_3$, Ag_3PO_4, CH_3COOAg, $Ag_2C_4H_4O_6$ and like, silver oxy-salts such as $Ag(Ag_3O_4)_2X$ were X can include but is not limited at NO_3^-, ClO_4^-, SO_4^{2-}, F^- etc. The term "medical device materials" may include materials such as metals, ceramics, glass, polymers, plastics, composite materials, a variety of natural materials, fabrics, textile made of either synthetic (HDPE, rayon, nylon, polyacetates, polyacrylics, glass etc.) or natural (cellulose, wool, jute, cotton, etc.) fibers.

The term "bacteriostatic activity", as used in this invention, relates to the inhibition of bacterial growth, but not actually killing the bacteria. Successful treatment therefore requires the host's immune system to clear the pathogen. Treatment is compromised when the antimicrobial materials are stopped before the pathogen has been completely cleared. The term "bactericidal activity", as used in this invention, relates to killing bacteria with or without lysis of the target cell. These types of antimicrobial materials are particularly advantageous in immunosuppressed individuals. A disadvantage to the bactericidal activity is cell lysis, which can release lipolysaccharides that are toxic to the host. However, if the concentration of the said antimicrobial material is relatively low, so that toxic effects cannot occur, a combination of both bacteriostatic and bactericidal activities would be ideal for antimicrobial materials.

The deposition of oxidized silver species is accomplished by first providing an aqueous solution of monovalent silver salt or silver complex such as silver nitrate, perchlorate, silver diamino complex, with silver nitrate being the most preferable if the reaction is carried out in the acidic or close to neutral conditions (i.e. at pH below 7) or silver diamino complex, i.e. $[Ag(NH_3)_2]^+$ being the most preferable if the reaction is carried out in alkaline conditions (i.e. at pH above 7).

Prior to coating, the article is preferably immersed in an alkaline solution containing 50 vol.% ethanol and 50 vol. % of an aqueous solution containing 30 g/L NaOH. Other cleaning and etching solutions can be used depending on the material of which the medical devise is made as well as on the toxicity of the said cleaning or etching solutions and on the possibility that some toxic substances may adsorb at the surface of the article to be coated. Of course any use of toxic or carcinogenic substances during the cleaning and etching processes should strictly be avoided. If coating is carried out in acidic solutions, the article is then carefully washed with distilled water until pH 7, in order to remove the residual alkali after the cleaning step.

When the reaction is carried out in the pH range below 7, the clean pretreated article to be used as a medical device containing oxidized silver species at the surface of

the same is simply immersed into an agitated 1 % $AgNO_3$ aqueous solution. After the exposure of the said medical device to the said silver solution under the agitation and acidic conditions (pH below 7) for duration from 2 to 10 minutes, a solution of an oxidizing agent is added. Although for this purpose a wide range of oxidizing agents such as permanganates, persulfates, hydrogen peroxide, hypochlorites etc., can be used under specific conditions and with the proper concentrations, the most preferred oxidizing agent in the present invention is ammonium persulfate. The amount of ammonium persulfate is crucial in order to allow the precipitation and deposition of oxide(s) and any oxidized silver species at the medical device. The concentrations of ammonium persulfate, according to the present invention may be in the range form 1 to 250 g/L with the concentration of about 50 g/L being the most preferable. After agitation for 2 to 5 minutes, the solution of 1 % $AgNO_3$ and ammonium persulfate is acidified with HNO_3, $HClO_4$ or CH_3COOH in a way that the concentration of the free acid is 9 % (HNO_3), 9 % $HClO_4$, 5 % (H_2SO_4) or 5 % (CH_3COOH). Although the use of these or other acids in the present invention can be avoided the most preferable acids in the present invention are H_2SO_4, $HClO_4$ or HNO_3. The agitation of the solution is not strictly required, in the present invention, however, in order to achieve a more uniform coating and more efficient reaction yield, the agitation of the solution is strongly recommended. This agitation can be realized by many different ways such as for example mechanical stirring, magnetic stirring or ultrasonic agitation. Upon addition of ammonium persulfate to the acidic solution of 1 % $AgNO_3$ within the time 1 to 10 minutes, depending on the concentration of ammonium persulfate as well as on the conditions of agitation, the formation of a yellow brown color of the solution and then a black grayish precipitate will occur. This brown color of the solution is attributed to the oxidation of Ag(I) to Ag(II). The black grayish deposit at the medical device or in the bulk solution is a consequence of the formation of silver oxy-salts such as $Ag(Ag_3O_4)X$, were X is an anion, depending on the acid used in the process e.g. HNO_3 (NO_3^-), H_2SO_4 (SO_4^{2-}), H_3PO_4 (PO_4^{3-}) etc. The decomposition of the silver oxy-salts may be presented as:

$$Ag(Ag_3O_4)_2X = AgX + AgO \quad (2)$$

Persulfates are powerful oxidizing agents. They can be reduced in the aqueous solutions, according to the following reactions:

$$S_2O_8^{2-} + 2e^- = 2SO_4^{2-}, \text{with } E^o = 1.96 \text{ V} \quad (3)$$

$$S_2O_8^{2-} + 2H^+ + 2e^- = 2HSO_4^-, \text{with } E^o = 1.96 \text{ V} \quad (4)$$

and

$$S_2O_8^{2-} + 2H_2O = 2H^+ + 2SO_4^{2-} + H_2O_2, \text{with } \Delta G^o = -36 \text{ kJ/mol} \quad (5)$$

A consequence of the reduction of persulfate is the oxidation of Ag(I) to Ag(II) and Ag(III), probably according to the following reactions:

$Ag^{+}=Ag^{2+}$ $+e^{-}$, with $E^{o}=1.98$ V (6)

$Ag^{+}+H_2O=AgO^{+}+2H^{+}+e^{-}$, with $E^{o}=1.998$ V (7)

$Ag^{2+}+H_2O=AgO^{+}+2H^{+}+e^{-}$, with $E^{o}=2.06$ V (8)

$Ag^{+}+H_2O=AgO+2H^{+}+e^{-}$, with $E^{o}=1.772$ V (9)

In this way the coated article to be used as a medical device may contain a combination of oxidized silver species i.e. Ag(I) – and Ag(II) – oxides as well as silver salts such as nitrates, persulfates, sulfates, phosphates, perchlorates and like and silver salts of a general formula $Ag(Ag_3O_4)_2X$ and may be traces of pure elemental silver. After coating, the sample is removed from the slurry and then carefully washed with distilled water until pH 7. When the washing is completed, the article is dried at room temperature and packaged.

When the reaction is carried out in the pH range above 7 (alkaline media) the article to be used as a medical device is the first immersed in an alkaline solution containing alcohol. The most preferable solution according to this invention is either NaOH or KOH with concentrations 15 to 40 g/L. The alcohol used in this solution may be ethyl alcohol, methyl alcohol or mixtures therein in a concentration above 50 vol.%. The immersion of the article to be used as a medical device into the alkaline solution containing alcohol is carried out in order to etch and clean the surface of the device to provide a reasonable adhesion of the film containing oxidized silver species which is deposited thereafter. The time of the immersion of the article is in the range from 5 to 20 minutes, with 10 minutes being the most preferable.

After the exposure to the alkali/alcohol solution for 10 minutes, the article is then removed without washing or rinsing into a second solution containing silver diamino complex i.e. $[Ag(NH_3)_2]^{+}$ in the concentration sufficient to adsorb silver ions at the surface of the article and for a duration of 2 to 5 minutes. This silver diamino complex is prepared from silver salt or silver oxide dissolved or suspended in water by a dissolution with NH_4OH (28 vol.%). Consequently, the silver diamino complex solution is prepared in a way that to a solution of any silver salt such as for example $AgNO_3$ or $AgClO_4$ or to any silver oxide e.g. Ag_2O or Ag_2O_2 or AgO or to any silver salt suspended in water e.g. AgCl, Ag_2CO_3, Ag_2SO_4 or like, the ammonium hydroxide is added in the proper concentration until a clear colorless solution is obtained. The concentration of silver ion in this silver diamino complex solution, as calculated for Ag^{+} ion can vary from 1 to 20 g/L with about 10 g/L being the most preferable. The pH of this solution is usually between 8 and 12 with the most preferred in the range 10 to 11.

After the exposure of the article to the second solution for 2 to 5 minutes, the article is removed without washing or rinsing into a third solution containing a strong alkali, most preferably NaOH or KOH. Under the agitation, the article is kept in this

solution until a clear colorless solution is obtained and the article is dyed with a tan, gray, brown or black color, depending on the desired amount of oxidized species to be deposited at the surface of the article. The time of contact of the article with the alkaline solution may vary depending on temperature and the silver ion concentration, but most preferable time is 1 to 15 minutes at room temperature or 1 to 10 minutes at temperatures 40 to 60 °C.

Alternatively, the process may involve an addition of the oxidizing agent solution e.g. $(NH_4)_2S_2O_8$. This solution is added directly to the alkaline solution containing the article. Depending on the amount of silver desired at the surface of the medical device, further additions of $Ag(NH_3)^+$ solution may also be required.

Upon immersion of the article previously exposed to an alkaline silver diamino complex solution to the aqueous solution of alkali, most preferably NaOH, the following reaction at the surface of the article may occur:

$$2Ag(NH_3)_2NO_3 + 2NaOH = Ag_2O + 4NH_3 + H_2O + 2NaNO_3 \quad (10)$$

In this way, at the surface of the article, Ag_2O will deposit as the result of the reaction (10). An addition of the oxidizing agent solution, i.e. $(NH_4)_3S_2O_8$ to the solution of NaOH containing the article the oxidation of silver ions and the reduction of $S_2O_8^{2-}$ ions will occur as presented with the following reactions:

$$Ag^+ = Ag^{2+} + e^-, \text{ with } E^0 = 1.96 \text{ V} \quad (11)$$

and

$$S_2O_8^{2-} + 2e^- = 2SO_4^{2-}, \text{ with } E^0 = 1.96 \text{ V} \quad (12)$$

The reactions of $Ag(NH_3)_2^+$ ion with the ammonium persulfate can be represented as follows:

$$Ag(NH_3)_2NO_3 + (NH_4)_2S_2O_8 = Ag_2S_2O_8 + 2NH_4NO_3 + 4NH_3 \quad (13)$$

$$Ag_2S_2O_8 + H_2O = 2AgO + 2H_2SO_4 \quad (14)$$

$$Ag(NH_3)_2NO_3+(NH_4)_2S_2O_8+2H_2O=2NH_4NO_3+2AgO+2H_2SO_4+4NH_3 \quad (15)$$

or

$$Ag(NH_3)_2NO_3+(NH_4)_2S_2O_8+2H_2O=2NH_4NO_3+2AgO+2(NH_4)_2SO_4 \quad (15)$$

In this way, the coating of the coated article may contain Ag_2O, AgO or other higher oxides of silver Ag(II), Ag(III) and mixtures therein. Also, if alcohol is present in the reacting solution, due to transferring from the etching solution some elemental silver may occur in the deposit. This is because in the presence of persulfates, alcohols can be oxidized to aldehydes according to the reactions:

$$CH_3OH = H_2CO + 2H^+ + 2e^- \quad (16)$$

$$C_2H_5OH = CH_3CHO + 2H^+ + 2e^- \quad (17)$$

Under the alkaline conditions, the aldehydes can reduce the silver ions to the elemental silver according to the reaction:

$$2Ag(NH_3)_2OH + HCHO = 2Ag + 4NH_3 + HCOOH + H_2O \quad (18)$$

After the coating of the article with the oxidized silver species is completed, article is removed, carefully washed with water until pH 7 and then dried at room temperature.

Following are the examples which illustrate the present invention.

EXAMPLES

Example 1

9 pieces of high density polyethylene gauze (HDPE), with dimensions 10 x 8 cm each, were immersed into a 100 ml solution containing 50 mL alcohol (95 % C_2H_5OH and 5 % CH_3OH) and 50 mL of 28 g/L NaOH solution for 5 minutes. This solution is in the further text referred to as etching solution. After 5 minutes of etching the HDPE gauze was transferred without washing or rinsing into 40 mL of an antimicrobial Ag^+ solution, containing 15.3 g $AgNO_3$ and a proper volume of NH_4OH (28 vol. %). The HDPE gauze was kept in this solution for 2 minutes. After 2 minutes of exposure to the ammoniacal $Ag(NH_3)_2^+$ solution, the HDPE gauze was transferred without washing or rinsing into 150 mL of 28 g/L NaOH solution stirred with a magnetic stirrer. As soon as the HDPE gauze was immersed into NaOH solution, the formation of a precipitate yellowish-brown in color occurred. Under the agitation the residual Ag(I) solution (about 38 mL) was added and after that 5 mL of 250 g/L $(NH_4)_2S_2O_8$ solution. The agitation was continued for the next 10 minutes. During this time the solution/precipitate became

black. The HDPE gauze was uniformly coated and it was black shiny in the appearance. The chemical analysis has found that HDPE gauze coated with oxidized silver species contained about 0.08 mg total silver per cm^2 of gauze. The coated HDPE gauze was then removed from the solution and carefully washed with distilled water until pH 7.00, and dried at room temperature. After drying, the gauze was a black – shiny in the appearance. The coated gauze was further analyzed by the XRD method. As found by the XRD analysis the gauze consisted of Ag_2O, Ag(II) oxides, $Ag(Ag_3O_4)NO_3$ and some traces of the elemental silver. Both bacterisostatic and bactericidal activities of silver coated HDPE substrates were tested against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. One hour bactericidal activity tests of coated HDPE gauze against both *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* were positive. The bacteriostatic activity was also tested. The controlled zone of inhibition, surrounding the test sample, were no bacteria growth occurred was estimated at 9 to 10 mm.

Example 2

HDPE gauze with dimensions 10 x 8 were immersed into a 100 mL of solution containing 50 mL of 28 g/L NaOH and 50 mL of denatured ethanol (95 % C_2H_5OH and 5 % CH_3OH) for 5 minutes. After 5 minutes of etching the HDPE gauze was transferred without washing or rinsing into a 40 mL of an ammoniacal Ag(I) solution containing 15.3 g/L $AgNO_3$ and a proper quantity of NH_4OH (28 vol. %). The HDPE gauze was kept under this solution for 2 minutes. The HDPE gauze was then transferred without washing or rinsing into a 150 mL of a solution containing 28 g/L NaOH. The NaOH solution immediately became brown. Upon addition of the residual Ag(I) solution (about 38 mL) the solution turned into a dark brown color and with a continued agitation for about 5 minutes the solution became black. After the agitation, the HDPE gauze appeared to be black. The HDPE gauze with a black color was clearly separated form the solution which was clear and colorless. When the agitation was stopped, the black precipitate occurred in the bulk solution as a result of its separation from the HDPE gauze. After washing and rinsing with distilled water the gauze appeared to be light tan or at the most slightly gray as a consequence of the coating with silver compounds. The amount of total silver deposited on the HDPE gauze, as determined by the chemical analysis was estimated at about 0.04 mg/cm^2. Antimicrobial activities (bactericidal and bacteriostatic) were tested against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. One hour bactericidal activity of coated HDPE gauze was positive. The bacteriostatic activity, as estimated according to the controlled zone of inhibition (CZOI) for the bacterial growth was also positive. The CZOI was estimated at about 4 mm.

Example 3

HDPE gauze was immersed into a 100 mL of 28 g/L NaOH solution for 5 minutes. The gauze was then transferred without washing or rinsing into a 40 mL of an

ammoniacal Ag(I) solution containing 15.3 g/L $AgNO_3$ and a proper volume of NH_4OH (28 %). After 2 minutes of immersion, the gauze was transferred without washing or rinsing into a 150 mL of 28 g/L NaOH solution stirred magnetically. The solution became immediately brown due to formation of a precipitate. A further addition of the residual Ag(I) solution (about 38 mL), has led to the formation of a dark brown precipitate. This color did not change even after 30 minutes of mixing at room temperature. The HDPE gauze which was exposed to this solution was then washed and rinsed very carefully with distilled water. The color of the HDPE gauze exposed to an alkaline Ag(I) solution according to this example did not change significantly. Although, it is important to note, that some change in color from white to a light tan appeared. The amount of total silver deposited on the HDPE gauze as described in this example is estimated at about 0.02 mg/cm^3. The antimicrobial activities (both bacteriostatic for the controlled zone of inhibition of the growth of bacterium and bactericidal) of these samples were tested against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. The results showed a positive bactericidal activity and the CZOI was estimated at about 3 mm.

Example 4

In a 250 mL beaker HDPE gauze was immersed into a 100 mL of a solution containing 1 g $AgNO_3$ and 1 mL of 67 % HNO_3. After 5 minutes of the immersion, 5 g of $(NH_4)_2S_2O_8$ dissolved in 20 mL of water was added. The sample was left for the next 30 minutes at room temperature. The solution with HDPE gauze was stirred occasionally with a glass rod. During this time the solution changed its color from colorless to a dark brown. Also a formation of a light gray precipitate in the bulk solution appeared. After 30 minutes, the HDPE gauze was removed from the slurry and carefully washed with distilled water. The washed HDPE gauze had a gray color. The coating was uniformly distributed at the surface of this material. The amount of total silver on HDPE gauze coated with oxidized silver species in a way described in this example is estimated at 0.09 mg/cm^2. The bactericidal activity for these samples was positive. The CZOI was estimated at about 8 mm.

Example 5

The HDPE gauze was coated with silver oxidized compounds in a way similar to that disclosed in the Example 4, with a few differences as outlined in the following text. The HDPE gauze was immersed into 100 mL of a solution containing 10 g/L $AgNO_3$ and 15 mL/L HNO_3 (67 %). To this solution 10 mL of 500 g $(NH_4)_2S_2O_8$ g/L was then added. The solution was magnetically stirred. After 7 minutes of stirring the solution became yellow-brown and formation of a very small amount of precipitate occurred. The stirring was continued for the next 30 minutes. After 30 minutes, the HDPE gauze was removed from the slurry and carefully washed with distilled water. The washed HDPE gauze had a gray color. The coating was uniformly distributed at the surface of this material. The amount of total silver on HDPE gauze coated with oxidized silver species

is estimated at 0.08 mg/cm^2. The bactericidal activities against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* were positive. The CZOI was estimated at about 7 mm.

Example 6

The HDPE gauze was coated with oxidized silver compounds in a way similar as described in the examples 4 and 5 with differences as described in the following text. The HDPE gauze was immersed into 100 mL of a solution containing 10 g/L $AgNO_3$ and 15 mL/L HNO_3 (67 %). To this solution 10 mL of 500 g $(NH_4)_2S_2O_8$/L was then added. The solution was agitated ultrasonically. After 2 minutes of stirring the solution became yellow-brown and formation of a very small amount of precipitate occurred. The stirring was continued for the next 30 minutes. After 30 minutes, the HDPE gauze was removed from the slurry and carefully washed with distilled water. The washed HDPE gauze had a gray color. The coating was uniformly distributed at the surface of this material. The amount of total silver on HDPE gauze coated with oxidized silver species is estimated at 0.08 mg/cm^2. The bactericidal activities against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* were positive. The CZOI was estimated at about 7 mm.

Examples 7, 8 and 9

In these examples the effect of different acids (anions) is clearly shown for coating of HDPE gauze with oxidized silver species under acidic conditions. As it was described in the Example 4, where HNO_3 was used, in the Examples 7, 8 and 9 perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4) and acetic acid (CH_3COOH) were used respectively. The HDPE gauze was immersed into a 100 mL of a solution containing 1 g $AgNO_3$. To this solution 1 mL of $HClO_4$ (70 %) (Example 7), 0.5 mL of H_2SO_4 (98 %) (Example 8) or 15 mL of CH_3COOH (5 %) (Example 9) was added. After 2 minutes of the exposure of HDPE gauze to these solutions, 20 mL of 250 g $(NH_4)_2S_2O_8$ /L was added. The mixing was continued for the next 30 minutes. In the solutions containing $HClO_4$ (Example 7) and H_2SO_4 (Example 8) formation of a black grayish precipitate occurred similarly as in the Example 4, where the nitric acid (HNO_3) was used. When the precipitate settled the solutions were clear and yellow-brown in color. This yellow-brown color suggests the presence of Ag(II) complexes in the solution.. The coated HDPE gauze was then removed from the slurry and carefully washed and rinsed with distilled water and thereafter dried at room temperature. After drying the gauze coated in the presence of 1 mL of $HClO_4$ (70 %) (Example 7), or in the presence of 0.5 mL of H_2SO_4 (98 %) (Example 8) appeared to be grayish in color. However, the HDPE gauze coated in the presence of 15 mL of CH_3COOH (5 %) (Example 9) was white and it did not change its color. The coated HDPE gauze (Examples 7, 8 and 9) were analyzed for the total silver content, and the antimicrobial activity was also evaluated against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. The amount of total silver on HDPE gauze coated with oxidized silver species is estimated at 0.08 mg/cm^2 (for samples coated in the presence $HClO_4$), 0.07 mg/cm^2 (for samples coated in the presence of H_2SO_4) and 0.01 mg/cm^2 (for the samples coated in the presence of CH_3COOH). The bactericidal activities against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* were positive. The CZOI was estimated at about 6 mm (for samples coated in the presence of

$HClO_4$ or H_2SO_4) while for samples coated in the presence of CH3COOH, the CZOI was estimated at 1 to 2 mm..

Example 10

HDPE gauze with dimensions 10 x 8 were immersed into a 100 mL of solution containing 50 mL of 28 g/L NaOH and 50 mL of denatured ethanol (95 % C_2H_5OH and 5 % CH_3OH) for 5 minutes. After 5 minutes of etching the HDPE gauze was transferred without washing or rinsing into a 40 mL of an ammoniacal Ag(I) solution containing 15.3 g/L $AgNO_3$ and a proper quantity of NH_4OH (28 vol. %). The HDPE gauze was kept under this solution for 2 minutes. The HDPE gauze was then transferred without washing or rinsing into a 150 mL of a solution containing 28 g/L NaOH. The NaOH solution immediately became brown. After mixing for 2 minutes, the solution became clear and colorless and the gauze was tan in color. When the agitation was stopped, the HDPE gauze was removed from solution and washed with distilled water. After washing and rinsing the gauze appeared to be tan in color as a consequence of the coating with silver compounds. The coated gauze was then analyzed for silver content and for antimicrobial activity against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. These samples contained between 0.04 and 0.08 mg/cm^2 total silver. The bactericidal activities against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* were positive. The CZOI was estimated at about 10 mm.

Example 11

A patterned wound dressing made of a perforated plastic carrier material with a skin adhesive layer comprised of a hydrophobic cross-linked silicon gel (trade name Mepitel, product of Mölnlycke, Sweden, dimensions 8 x15 cm) was exposed to a solution containing 15 g/L NaOH and 500 mL/L denatured ethanol (95 % C_2H_5OH and 5 % CH_3OH) at room temperature for 5 minutes. Under the conditions of agitation 40 mL of a solution containing 15.3 g/L $AgNO_3$ and a proper volume of NH_4OH (28 vol.%) was added. The Mepitel was kept under solution and agitation was continued for the next 5 minutes. The Mepitel was then removed from the solution and carefully washed with distilled water. The sufficient drops of water were removed with a soft paper and the Mepitel was dried at room temperature. The coated Mepitel was further analyzed for antimicrobial activity against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. MH plates and Tryptic Soy Broth were used for analysis. *Pseudomonas Aeruginosa* standard was set to 0.5 Mc Farland standard. One hour of the bactericidal activity of coated Mepitel against tested bacteria where TSB broths were incubated for 24 hours was positive. The controlled zone of inhibition (CZOI), for the bacterial growth (bacteriostatic activity), were above 8 mm. Furthermore, the very same samples of coated Mepitel were tested for seven days for the antimicrobial activity. The values of CZOI after 2 days were 20.5 mm, after 3 days 19 mm, after 4 days 20.5 mm, after 5 days 19 mm and after 7 days 7 mm. This shows a very good resistance towards bacteria for a relatively long time (7 days).

Any scientific publications, information, patents or applications referred to herein are incorporated by reference in their entirety. The terms and expressions in this specification are used as terms of description and not of limitation. The scope of the invention is defined and limited only by the following claims.

I claim,

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